

FIG. 1

cvHAS	MG--KNIIIM	VSUWYTIITS-	-----NL	IAGVGASLIT	APAITGVVLH	39
seHAS	MRTLKNLIT-	-----	-----V	VAFSIFWVL	I-----VNV	25
spHAS	VPIFKKTLI-	-----	-----V	LSFIFLISIT	I-----LNM	25
huHAS	MHCERFLCIL	RI---IGTTL	-----	---FGVSL	LGITAAIVG	33
xlHAS	MK-EKAAETM	EIPEGIPKDL	EPKHPTLWRI	IYYSFGVVL	ATITAAVVAE	49
cvHAS	WNIALST--I	WGVSAVGFV	FGFFLAQVLF	SELNRKRLRK	WISLRPKGWN	87
seHAS	YLFAGK---	-SLSIYGFLL	IAYLLVKMSL	SFF-YKPFKG	R---AGQ--Y	65
spHAS	YLFGT-S---	-TVGIYGVIL	ITYLVIKLGL	SFL-YEPFKG	N---PHD--Y	64
huHAS	YQFIQTDNYI	FSFGLYGAF	ASHLIIQSIF	AFLEHRKMCK	SLETPIK--L	81
xlHAS	FQVLKHEAIL	FSLGLYGLAM	LLHLMQSLF	AFLEIRRVNK	S-ELPCS--F	96
cvHAS	DVRLAVITAG	VREDPYMFQK	CEFSVRDS	GNVA-RLICV	IDGDEDDDMR	136
seHAS	K--VAALIPS	YNDAESLLE	TEKSVQQT	PLAE--IYV	DDGSADETI	111
spHAS	K--VAAVIPS	YNDAESLLE	TKSVLAQT	PLSE--IYV	DDGSSNTDAI	110
huHAS	NKTVLCTAA	YQEDPDYLRK	CEOSVKRLT	PG--IKVVM	IDGNSEDDLY	129
xlHAS	KKTVALTTAG	YQENPEYLIK	CEESCKYVK	PKDKLKIIL	IDGNTEDDAY	146
cvHAS	MAAVYKAIYN	DN-----	-----IKKPE	-----FV	LCESDDKEGE	165
seHAS	KR-----	---IEDYVRD	-----	TGDLSSNVIV	HRSEKNQGR	140
spHAS	QL-----	---IEEYVNR	-----	EVDICRNVIV	HRSLVNKGGR	139
huHAS	MMDIFSEVMG	RDKSATYIWK	NNFHE-KGPG	ETDES-----	-----HKESS	168
xlHAS	MMEMFKDVFH	GEDVGTYVWK	GNHTVKKPE	ETNKGSCPEV	SKPLNEDEGI	196
cvHAS	RIDSDF---S	RDICVLOPHR	GKRECLYTG	QLAKMDPSVN	AVVLISSDTV	212
seHAS	HA-----	---QAW-	-----A	E--RSDADV-	FLTV-SSDTY	163
spHAS	HA-----	---QAW-	-----A	E--RSDADV-	FLTV-SSDTY	162
huHAS	QHVTQLVLSN	KSICIMQKWG	GKREVMYTA	R--ALGRSVD	YVQVCSDDTM	216
xlHAS	NMVEELVRNK	RCVCIMQWVG	GKREVMYTA	Q--AIGTSVD	YVQVCSDDTK	244
cvHAS	LEKDAILEVV	YPLACDPEIQ	AVAGECKIWN	T-DTLLSLLV	AWRYYSACV	261
seHAS	IYPDALEELL	KTFNDPTVFA	ATG-HLNVNR	RQTNLLTRLT	DIRYDNAGV	212
spHAS	IYPNALEELL	KSFNDETIVA	ATG-HLNVNR	RQTNLLTRLT	DIRYDNAGV	211
huHAS	LDPASSVMV	KVLEEDPMVG	GVGGDVQILN	KYDSWISFLS	SVRYWMAFNI	266
xlHAS	LDELATVEMV	KVLESNDMYG	AVGGDVRIIL	PYDSFISFMS	SLRYWMAFNV	294
cvHAS	ERSAQSFFRT	VQCVGGPLGA	XKIDIKEIK	DPWISORL	QKCTYGDHRR	311
seHAS	ERAADSVTGN	ILVCSGPTSV	YRREVVPNI	DRYINOTL	IPVSIEDDRC	262
spHAS	ERAASLTGN	ILVCSGPTSI	YRREVIIPNL	ERYKNOTL	LPVSIEDDRC	261
huHAS	ERACQSYFGC	VQCISGPTGM	YRNSLLHEFV	EDWYNQCEMG	NQCSFGDDRH	316
xlHAS	ERACQSYFDC	VSCISGPTGM	YRNILQVFL	EAWYRQKEL	TYCTLGDDRH	344
cvHAS	LTNEILMRK	KVVFTPPAVG	WSDSETNVFR	YIVQOTRWS	SWCRBIWYTL	361
seHAS	LTNYATDLG	KTVYQSTAKC	ITDVPDKMST	YLKQONRWNK	SEFFRESIISV	311
spHAS	LTNYAIDL	RTVYQSTARC	DTDVPQOLKS	YLKQONRWNK	SEFFRESIISV	310
huHAS	LTNRVLSLGY	ATKYTARSKC	LTETETIEYLR	WLNQOTRWS	YFREWLYNA	366
xlHAS	LTNRVLSMGY	RTKYTHKSRA	FSETESLYLR	WLNQOTRWS	YFREWLYNA	394
cvHAS	FAAWKHGLSG	IWLAFECYQ	ITYFFLVYIL	FSRLAVEADP	RAQTATVIVS	411
seHAS	KKIMNPPFVA	LWILEVSMF	MMLVYSVVDF	FVGNVREFDW	LRVLAFLVII	361
spHAS	KKILSNPIVA	LWITFEVVMF	MMLIVAIGNL	LFNQAIQLDL	IKLFAFLSII	360
huHAS	MWFHKHH---	LWMTYEAIT	GFFPFFLIAT	VIQLFYRGKI	WNILLFLLTV	413
xlHAS	QWWHKHH---	IWMTYESVVS	FIFPFFITAT	VIRLIYAGTI	WNVVWLLLCI	441
cvHAS	TTVAIIKCGY	FSFRAKDIRA	FYFV-LYTFV	YFFCMIPARI	TAMTLWDIG	460
seHAS	FIVACRNHIH	YM--LKHPLS	FLLSPFYGV	HLFVLOPLKL	YSLFTIRNAD	409
spHAS	FIVACRNH	YM--VKHPAS	FLLSPLYGIL	HLFVLOPLKL	YSLCIIKNT	408
huHAS	QLVGLIKSS-	FASCLRGIV	MVFMSTSVL	YMSLLPAKM	FAIATINKAG	462
xlHAS	QIMSIFKSI-	YACWLRGNFI	MLLMSLYSML	YMTGLLESKY	FALLTINKTG	490
cvHAS	WDTRGGNEKP	SVGTRVALWA	KQYLIAYMWW	AAVVGAGVYS	IVHNMWFDWN	510
seHAS	IG-----	RRK L-----	-----	-----	-L*	417
spHAS	IGT-----	RRK V-----	-----	-----	T IFK*	419
huHAS	IGTSG--RKT	IVNFIGL--	---IPVSVWF	TILLGGVIFT	IYKESKRPF	505
xlHAS	IGTSG--RRK	IVGNYPMI--	---LPLSIWA	AVLCGGVGYS	IYMDQNDWS	533
cvHAS	S-----LSYR	FALVGIC-SY	IVFIVIVLVV	YFTGKITWN	FTKLQKELIE	554
huHAS	ES-KQTVLIV	GTLTYAC---	--YWMVLLTL	YV---VLINK	CGRKKKGQY	546
xlHAS	TPEKQKEMY-	-HLLYGCYGY	VMYVWIMAVM	YW---VWVKR	CCR-KRSQTV	577
cvHAS	DRVLYDATTN	AQSV*				568
huHAS	DMVL----	DV*				552
xlHAS	TLVH----	DI PDMCV*				588

FIG. 2

Phylogenetic tree showing the relationships between various HAS sequences. The tree is rooted on the left and branches to the right. The sequences listed on the left are: seHAS, spHAS, muHAS2, huHAS2, muHAS3, muHAS1, huHAS1, and xlHAS. Bootstrap values are indicated at the nodes: 67.2% for the seHAS/spHAS node, 98.7% for the muHAS2/huHAS2 node, 94.1% for the muHAS1/huHAS1 node, 58.0% for the muHAS2/huHAS2 node joining the muHAS3 branch, 46.6% for the muHAS1/huHAS1 node joining the xlHAS branch, 40.9% for the muHAS2/huHAS2/huHAS1 branch joining the muHAS3 branch, and 10.7% for the final root node joining the seHAS/spHAS branch.

Sequence	Bootstrap Value (%)
seHAS	67.2
spHAS	67.2
muHAS2	98.7
huHAS2	98.7
muHAS3	40.9
muHAS1	94.1
huHAS1	94.1
xlHAS	46.6
Root	10.7

FIG. 3

SIZE DISTRIBUTION OF HYALURONAN  
PRODUCED BY DIFFERENT ENGINEERED  
STREPTOCOCCAL HAS ENZYMES

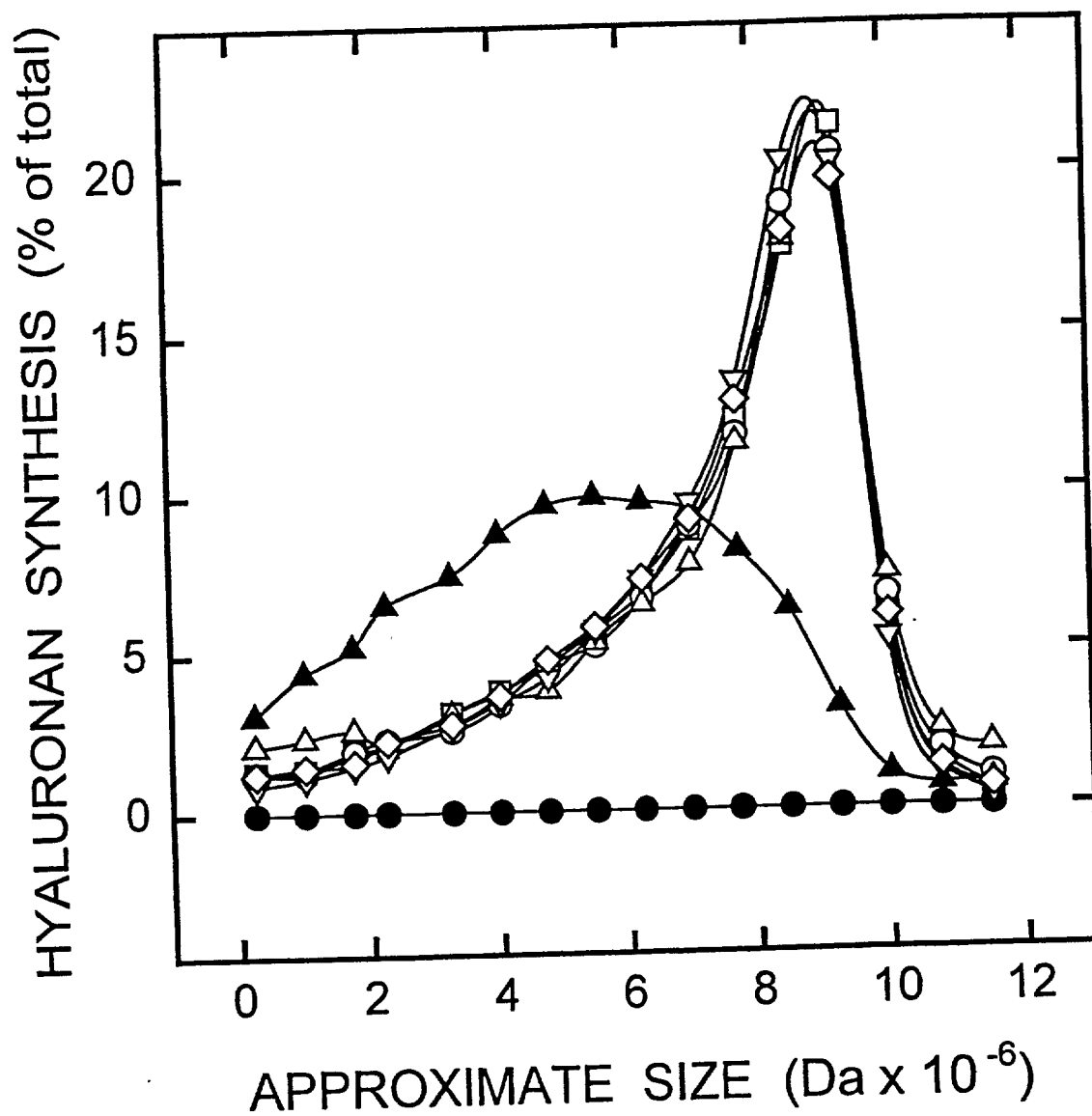


FIG. 4

092992860

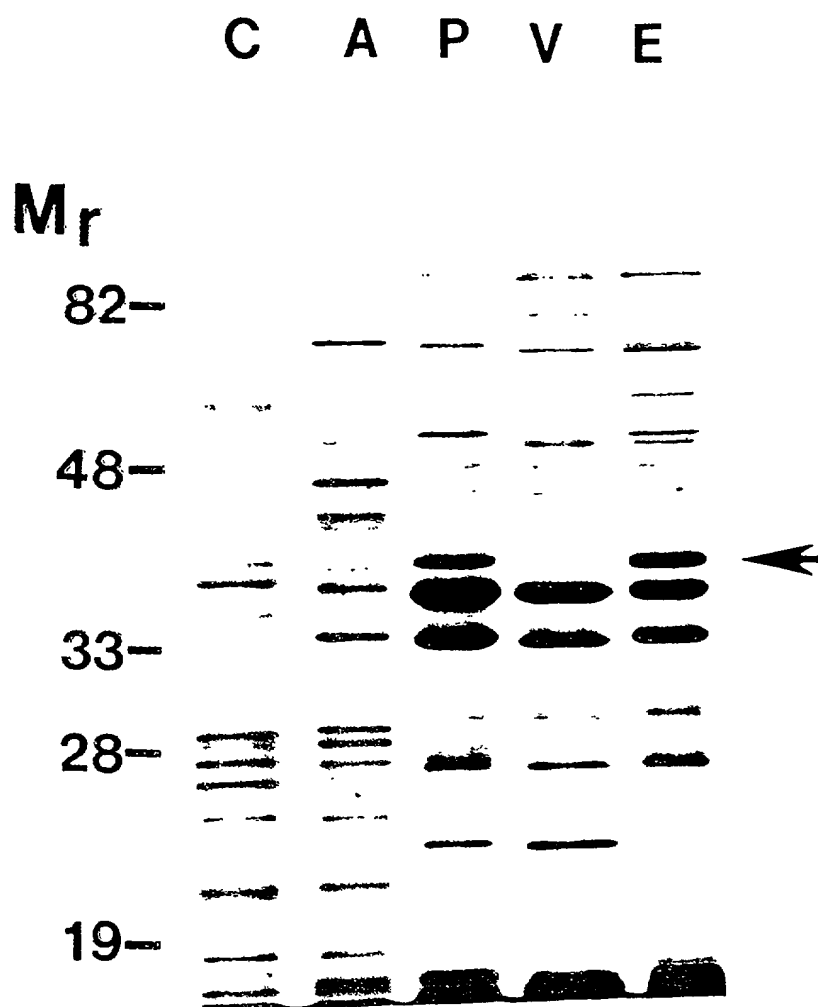


FIG. 5

SDS-PAGE gel image showing protein bands across 10 lanes. Molecular weight markers are indicated on the left at 200, 116, 66, 55, 36, and 31 kDa. Lane 1 is a molecular weight marker. Lanes 2-10 show various protein bands, with prominent bands at approximately 116, 66, 55, 36, and 31 kDa.

FIG. 6

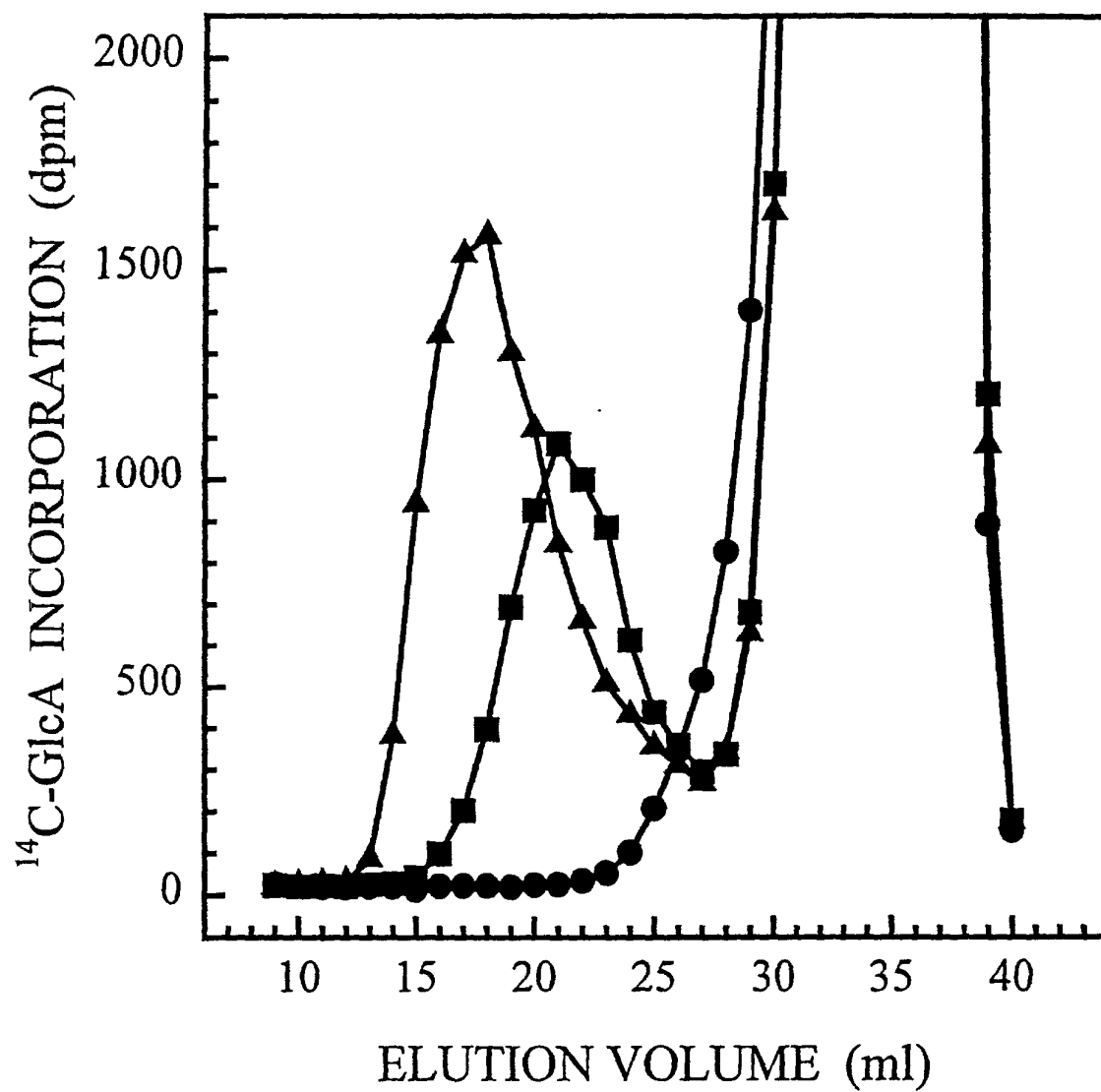


FIG. 7

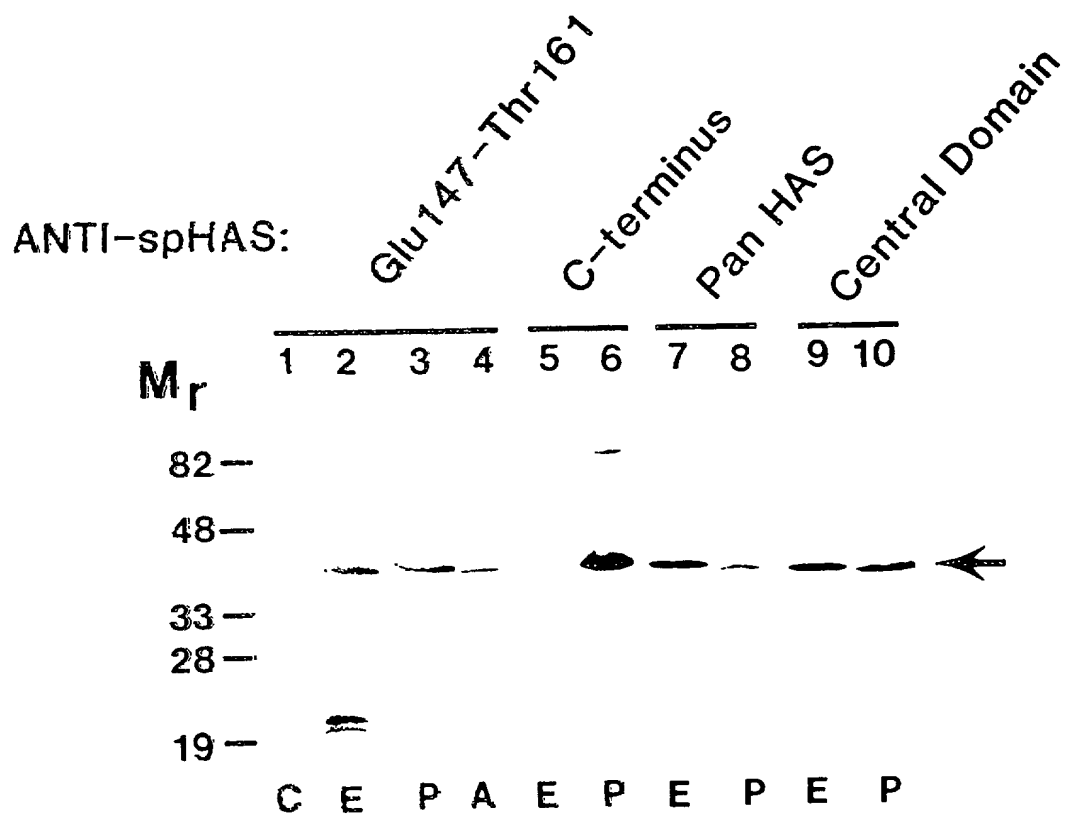


FIG. 8



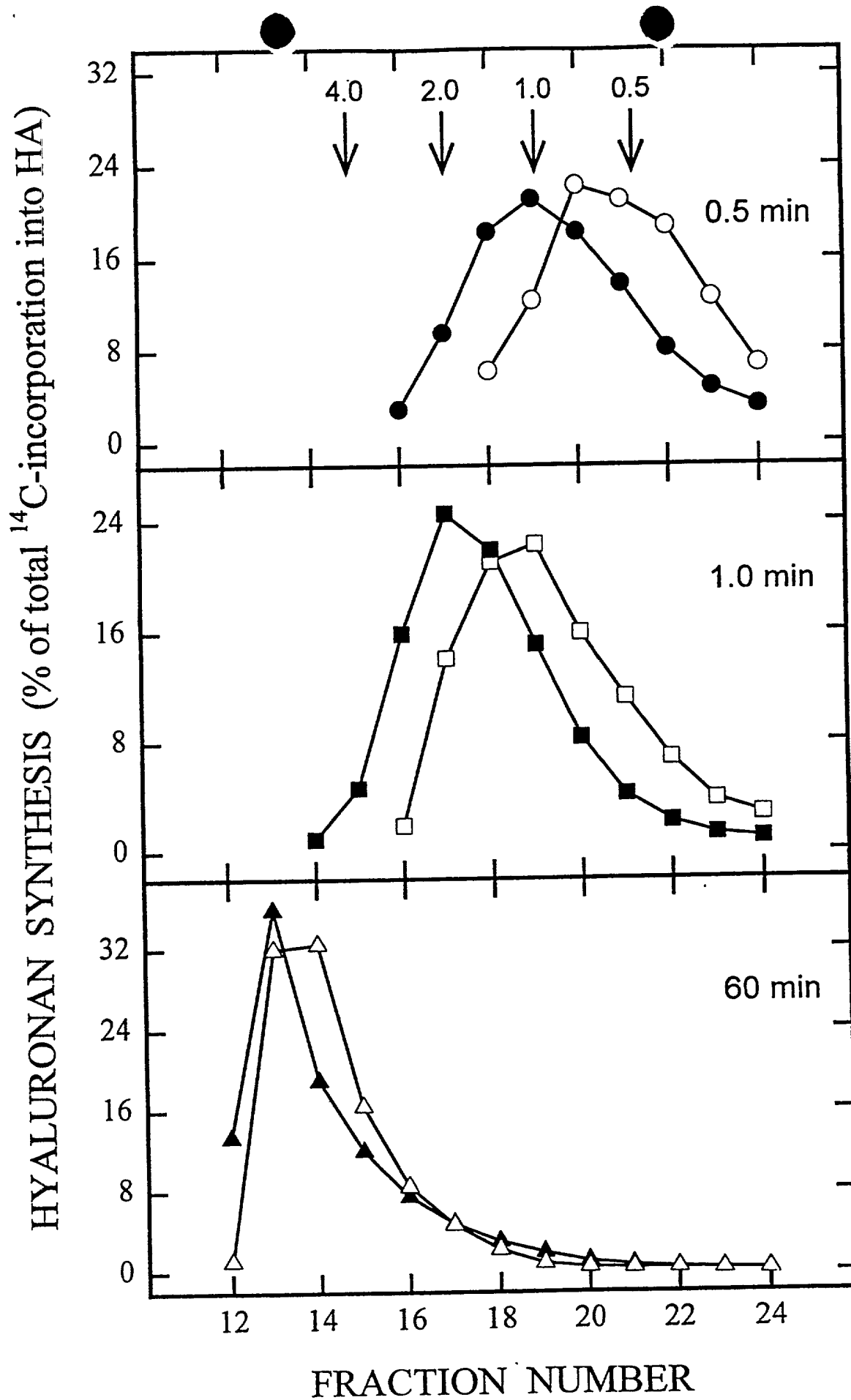


FIG. 9

TEET90" 6566/850

HYDROPHILICITY

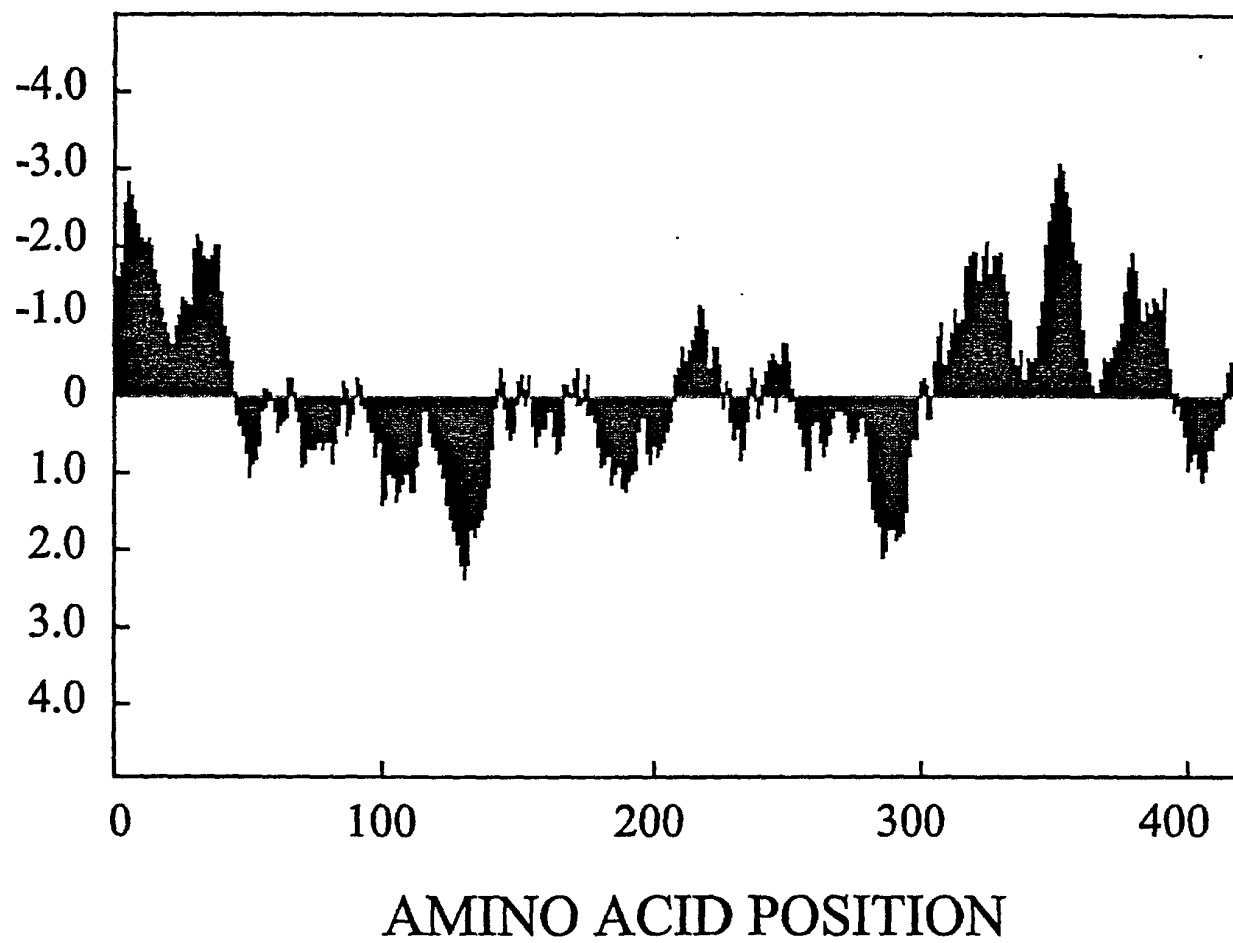


FIG. 10

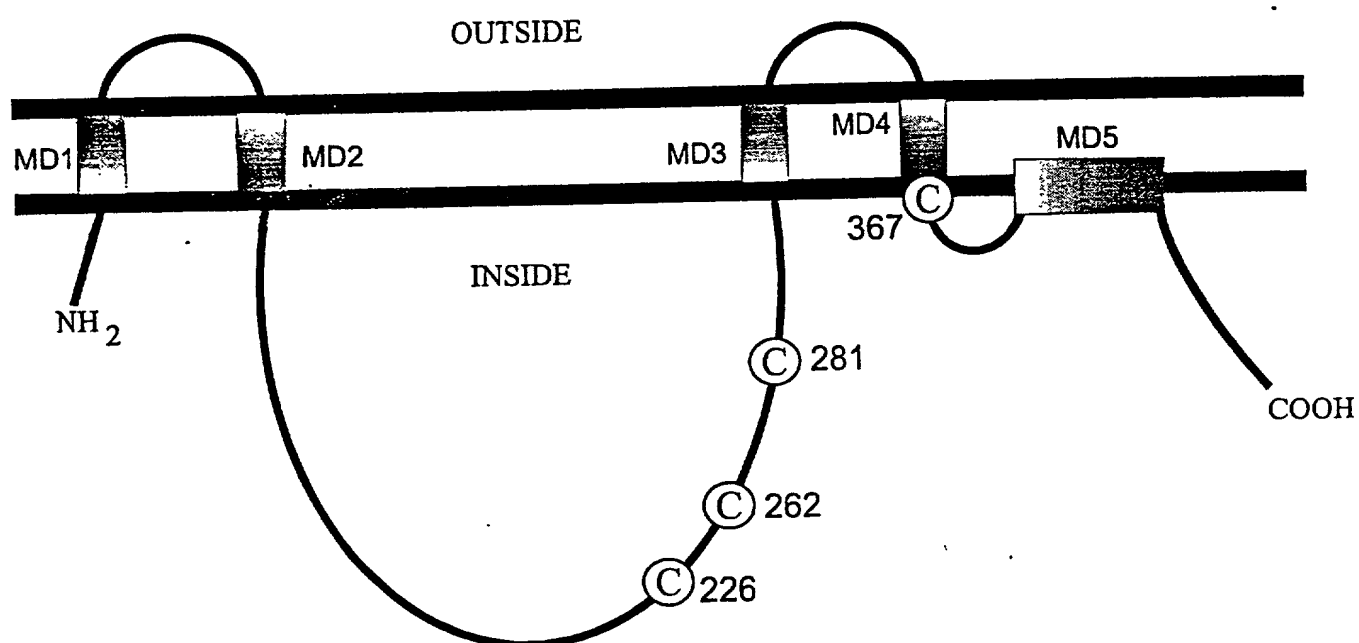


FIG. 11

Figure 1 consists of two vertically stacked chromatograms, A and B, sharing a common x-axis representing elution volume in milliliters (ml), ranging from 0 to 45 ml with major ticks every 10 ml. Chromatogram A shows the elution of [<sup>14</sup>C]glucuronic acid (dpm) on the y-axis, ranging from 0 to 20,000 dpm. It features three distinct peaks: a small peak at V<sub>0</sub> (~15 ml), a medium peak at BD (~20 ml), and a large peak at V<sub>i</sub> (~35 ml). The peaks at V<sub>0</sub> and BD are marked with solid black circles, while the peak at V<sub>i</sub> is marked with open circles. Chromatogram B shows the elution of [<sup>3</sup>H]N-acetylglucosamine (dpm) on the y-axis, ranging from 0 to 36,000 dpm. It also features three distinct peaks: a small peak at V<sub>0</sub> (~15 ml), a medium peak at BD (~20 ml), and a large peak at V<sub>i</sub> (~35 ml). The peaks at V<sub>0</sub> and BD are marked with solid black squares, while the peak at V<sub>i</sub> is marked with open squares. Arrows at the top of the x-axis indicate the positions of V<sub>0</sub>, BD, and V<sub>i</sub>.

FIG. 12

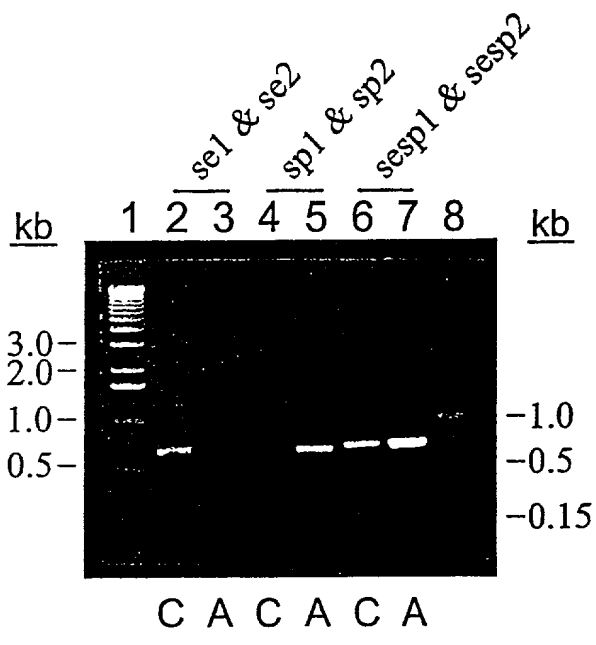


FIG. 13